Role of cholinergic anti-inflammatory pathway in regulating host response and its interventional strategy for inflammatory diseases

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The cholinergic anti-inflammatory pathway (CAP) is a neurophysiological mechanism that regulates the immune system. The CAP inhibits inflammation by suppressing cytokine synthesis via release of acetylcholine in organs of the reticuloendothelial system, including the lungs, spleen, liver, kidneys and gastrointestinal tract. Acetylcholine can interact with α7 nicotinic acetylcholine receptors (α7 nAchR) expressed by macrophages and other cytokine producing cells, down-regulate pro-inflammatory cytokine synthesis and prevent tissue damage. Herein is a review of the neurophysiological mechanism in which the CAP regulates inflammatory response, as well as its potential interventional strategy for inflammatory diseases.

Key words: ***Cholinergic agents; Vagus nerve; Inflammation; Alpha-bungarotoxin receptor***

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Adequate inflammatory response initiated by infection and injury can eradicate the invading pathogen and enhance wound healing. If the inflammatory response is excessive, it will cause cytokine overproduction which induces general tissue damage and organ dysfunction. Excessive cytokine production and release are characteristics of chronic or uncontrolled inflammatory responses and primarily related to the pathology of many diseases, including sepsis, rheumatoid arthritis, Crohn's disease and other autoimmune diseases.[1] Thus, inhibiting pro-inflammatory cytokines is beneficial to prevent the occurrence and progress of uncontrolled inflammatory responses. For instance, experimental therapies that neutralize pro-inflammatory cytokines, including monoclonal anti-tumor necrosis factor (TNF) antibodies, interleukin (IL)-1 receptor antagonists and TNF-receptor fusion proteins, are successfully used in rheumatoid arthritis, Crohn's disease, ankylosing spondylitis and psoriasis. Recent studies have identified high mobility group box 1 protein (HMGB1) as an important late inflammatory mediator in sepsis and several inflammatory disorders. Anti-HMGB1 therapeutics are successful in reducing multi-organ injuries and improving survival in inflammatory disease models.[2]

Anti-inflammatory mechanisms include the release of glucocorticoids, anti-inflammatory cytokines such as IL-10 and transforming growth factor-beta (TGF-β), and soluble receptors which neutralize the activity of cytokines. Recent studies found that vagus nerve-mediated cholinergic anti-inflammatory pathway (CAP) can effectively regulate systemic inflammatory response. This article reviewed the neurophysiological mechanism in which the CAP regulates inflammatory response, as well as its potential interventional strategy for inflammatory diseases.

Cholinergic anti-inflammatory pathway

The vagus nerve, in addition to its classically assigned function of controlling heart rate, hormone secretion, gastrointestinal peristalsis and digestion, may also be involved in regulating pro-inflammatory cytokines (e.g. TNF-α) release. Even when circulating cytokine levels are low, vagus nerve can detect peripheral inflammation. Inflammatory signals to central nervous

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system *via* afferent vagus nerve activate CAP mediated by efferent vagus nerve, which regulates cytokine production and balances inflammatory response (Fig.1).

Peripheral inflammatory mediators activate afferent vagus nerve signals,[3] which are transmitted to medulla, locus ceruleus, hypothalamus and dorsal motor nucleus of vagus. Then adenohypophysis secretes adrenocorticotriphic hormone (ACTH), which increases systemic glucocorticoid levels so as to inhibit pro-inflammatory cytokine synthesis. Meanwhile, melanotropin induced by inflammation can also suppress cytokine synthesis. Ascending sensory fibers of the vagus nerve synapses in the nucleus tractus solitaries (NTS) and preganglionic vagus nerve fibers, that is CAP,[4] are originated from dorsal motor nucleus (DMN). Anti-inflammatory signals in vagal efferent nerve fibers arrive at the reticuloendothelial system, including the spleen, liver, heart, kidneys and gastrointestinal tract. Acetylcholine released from vagus interacts with α 7 nicotinic acetylcholine receptors (α 7 nAchR) expressed by macrophages and other cytokine producing cells to inhibit cytokine production. The intracellular mechanisms in which cholinergic stimulation blunts cytokine production are not completely clear, but current study suggests that the CAP acts at both the transcriptional and post-transcriptional levels *via* Janus kinase (JAK) signal transducer and activator of transcription (STAT) and nuclear factor kappa B (NF-κB) pathways.

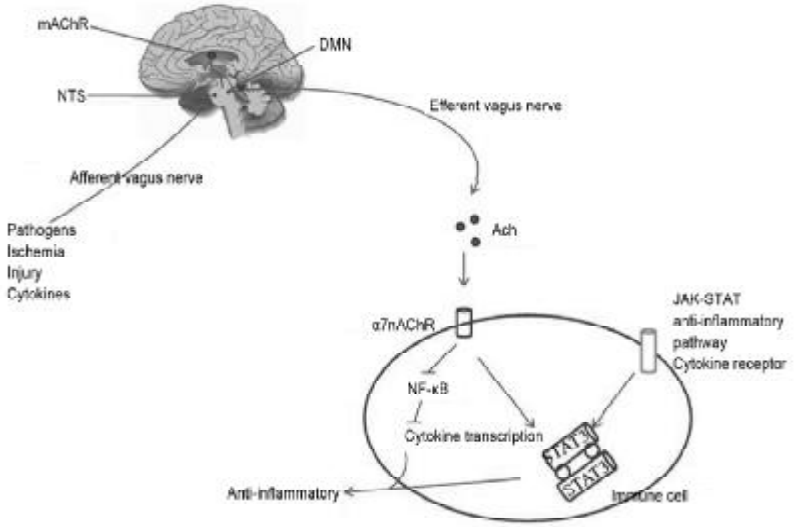

Fig. 1. The cholinergic anti-inflammatory pathway.

Given the short half-life of acetylcholine, cholinergic modulation of immune cell activation most likely requires close contact. Although macrophages are found in close anatomical apposition to cholinergic fibers in the intestine and spleen, there is no evidence that parasympathetic neurons indeed innervate macrophages.[5] It remains to be elucidated whether acetylcholine released from vagus nerve termini actually reaches the immune cells, and if so, in what quantities.

Components of the cholinergic anti-inflammatory pathway

Vagus nerve Vagus nerve-derived cholinergic signals provide continuous neurological modulation of cytokine synthesis to limit the magnitude of the immune response. TNF-α, a major inflammatory mediator in endotoxemic hypotension, could be markedly inhibited during endotoxemic shock in rats by vagus nerve stimulation (VNS).[6] Consistently, surgical or chemical vagotomy can repress the activity of vagus nerve, which may lead to the increase of TNF-α release and septic shock. Thus, vagus could be identified as a physiological anti-inflammatory system controlling immune reaction.

Vagus nerve signaling inhibits cytokine activities and improves disease endpoints in experimental models of sepsis, ischemia/reperfusion injury, hemorrhagic shock, myocardial ischemia, ileus, experimental arthritis and pancreatitis. However, VNS does not stimulate an increase in either corticosteroid or IL-10 responses. Therefore, anti-inflammatory effects of vagus nerve could not be attributed to anti-inflammatory humoral mechanisms. Interestingly, the dissociability and lower

activation threshold of vagal anti-inflammatory signaling suggest that the A fibers, which have the lowest activation threshold and do not appear to participate in heart rate regulation, may play the role of cholinergic anti-inflammatory fibers.[7]

Splenic nerve Catecholaminergic nerve terminals are found adjacent to splenic macrophages in the red pulp and the marginal zone which are the major sources of splenic TNF-α during endotoxemia. Surgical ablation of the splenic nerve and catecholamine depletion by reserpine abrogate the TNF-suppressive effect of VNS, which indicates that splenic nerve are required for functional inhibition of TNF-α production by VNS. Thus, the CAP regulates TNF-α production in discrete macrophage populations *via* two serially connected neurons: one preganglionic, originating in the dorsal motor nucleus of the vagus nerve, and the other postganglionic, originating in the celiac superior mesenteric plexus, and projecting in the splenic nerve.[8] It is possible that acetylcholine released by the vagus nerve acts on α7 expressed in the ganglia of the celiac superior mesenteric plexus to modulate splenic nerve function, playing an anti-inflammatory role. Furthermore, acetylcholine may be released from spleen by stimulation of the splenic nerve. So it is possible that catecholaminergic signals *via* the splenic nerve enhance acetylcholine levels in the spleen, which attenuate cytokine production by signaling through α 7 nAchR.

Spleen The spleen is an important source of systemic TNF-α following septic challenge. VNS could inhibit serum TNF-α levels but fails to inhibit TNF-α formation in splenectomized animals during lethal endotoxemia. In septic animals, administration of nicotine that mimics vagus nerve stimulation, decreases pro-inflammatory cytokine production and lethality, while splenectomy could reverse the protective effects of nicotine. These findings indicate that the spleen is critical to the protective response of the cholinergic pathway.[9] Selective lesion of the common celiac nerve abolishes TNF-α suppression by VNS, suggesting that the cholinergic pathway functionally links to the spleen *via* this branch of the vagus nerve.

α 7 nAchR nAChR is composed of five receptor subunits, including α1-α10, β1-β4, γ, δ and ε, which form ligand-gated ion channels.[10] According to their physiological distribution, nAChRs are classified as muscle nAChRs (consisting of α 1, β 1, γ, δ, and ε subunits) or neuronal nAChRs (consisting of α2-α10 and β2-β4). The neuronal nAChRs are further subclassified into either homomeric nAChRs (e.g. α7 or α9) or heteromeric nAChRs (including combinations of α and β subunits, e.g. α3β2). In neurons, α 7 nAChR assembles as a homopentamer composed of five individual α7-subunits which form a central pore with ligand binding at subunit junctions responsible for changes in the state of the receptor. α 7 homopentamer is highly permeable for Ca^{2+} in preference to Na^{+} and has low sensitivity to acetylcholine. The identity of the α7 subunit expressed on macrophages is confirmed by cloning and sequencing, and is identical to the α 7 subunit expressed in neurons. Western blot analysis reveals that the α 7 subunit protein expressed in macrophages has a relative molecular mass of 55 kDa, identical to that described in neurons. However, whether the structure and functional activity of non-neuronal α 7 nAChR is the same as that of neuronal α 7 nAChR remains to be elucidated.

It has been reported that acetylcholine attenuates the release of cytokines (TNF-α, IL-1β, IL-6 and IL-8), but not the anti-inflammatory cytokine IL-10, in lipopolysaccharide (LPS)-stimulated human macrophage cultures.[6] The effects of acetylcholine are mediated by α-bungarotoxin-sensitive nAChR, which is confirmed as α 7 nAChR by subsequent studies.[11] VNS inhibits TNF-α synthesis in wild-type mice, but fails to inhibit TNF-α synthesis in α 7-deficient mice. Thus, α 7 subunit is essential for inhibiting cytokine synthesis by the CAP.[11] This viewpoint is further confirmed by the fact that VNS inhibits TNF-α synthesis without the involvement of muscarinic receptors in immune cells.[12]

α 7 receptor is widely expressed in endothelial cells, enterocytes, T lymphocytes, B lymphocytes, dendritic cells, monocytes, macrophages, neutrophils and microglia cells. Therefore, α 7 receptor agonists have systemic anti-inflammatory effects on various organs and tissues. Nevertheless, VNS acts through α 7 nAChRs on macrophages, there are no data demonstrating that α 7 receptors on other cell types are regulated by vagus nerve.

Central muscarinic receptor Intracerebroventricular administration of muscarine receptor agonists signifi-

cantly reduces serum TNF-α levels during endotoxemia. It also increases instantaneous heart rate variability, an indicator of enhanced vagus nerve activity in the periphery. These observations indicate that muscarinic brain networks regulate the cytokine-controlling function of vagus nerve. Vagus nerve fiber could be stimulated by CNI-1493, a tetravalent guanylhydrazone molecule that down-regulates systemic inflammation. Anti-inflammatory effects of CNI-1493 are also mediated by central muscarine receptors. In addition, galantamine, an acetylcholinesterase inhibitor, could pass through blood-brain barrier, inhibit brain acetylcholinesterase and thus facilitate brain cholinergic transmission. Galantamine suppresses systemic inflammation during endotoxemia through a central muscarinic receptor-mediated and vagal- and α7 nAChR-dependent mechanism.[12]

Controlling inflammation through the cholinergic anti-inflammatory pathway

Electrical VNS and/or α7 nAChR agonists have been successfully applied in models of inflammatory diseases, including endotoxaemia, sepsis, ischaemia/reperfusion injury, haemorrhagic shock, subcutaneous and gastrointestinal inflammation and pancreatitis.

Sepsis VNS can reduce excessive inflammation and acute reaction to sepsis. During lethal endotoxaemia, VNS inhibits TNF-α synthesis in liver, attenuates peak serum TNF-α levels and prevents the development of shock. In contrast, vagotomy significantly increases the levels of serum and liver TNF-α.[6] VNS can also prevent the development of cecal ligation and puncture (CLP)-induced hypotension, alleviate hepatic damage and reduce the plasma TNF-α release. Moreover, transcutaneous VNS reduces systemic TNF-α levels during lethal LPS challenge, inhibites HMGB1 levels and improves survival in mice with polymicrobial sepsis.[7]

Anti-inflammatory effects of agonists for α7nAChR in experimental sepsis have also been confirmed. Nicotine, a highly efficient nAChR agonist, inhibits HMGB1 release of macrophages induced by either LPS or TNF-α *via* activation of α7nAChR; *in vivo*, nicotine treatment attenuates serum HMGB1 levels and improves survival in CLP models. These effects can be attributed to acute reduction of HMGB1 and pro-inflammatory cytokines, rather than effects on bacterial outgrowth when mice receive nicotine 24 hours after CLP.[13] Another report reveals that experimentally induced systemic inflammation using endotoxin in other healthy human volunteers can be reduced by pretreatment with transcutaneous nicotine.[14] Cotinine is a stable nicotine catabolite. Pretreatment of monocytes with cotinine shiftes the inflammatory response to LPS to an IL-10-dominated anti-inflammatory profile which is initiated by engagement of the monocytic α7 nAChR.[15] GTS-21, a selective α7 nAChR agonist, significantly inhibits serum HMGB1 levels in CLP mice and improves survival. Also, GTS-21 strongly inhibits LPS-induced TNF-α release into the peritoneal cavity and attenuates the influx of neutrophils into peritoneal fluid upon administration of LPS, and it is independent of its effect on TNF-α release. Choline is a precursor in the biosynthesis of acetylcholine and a selective natural α7 nAChR agonist. Recent study has shown that choline can suppress TNF-α release from LPS-activated macrophage-like cells, reduce systemic TNF-α levels during endotoxaemia through an α7 nAChR-mediated mechanism and suppress HMGB1 release in mice with severe sepsis. The anti-inflammatory effect of vagus nerve in lungs is limited, however, anti-inflammatory effect of α7nAChR agonists in lungs is obvious. For example, nicotine, choline and PNU-282987 (a specific α7agonist) reduce acid-induced acute lung injury; local administration of GTS-21 inhibits TNF-α release in lungs during LPS-induced sepsis.

Interestingly, vagus nerve firing and α7nAChR activation have a detrimental effect on host defense against bacteria in septic animals. Pretreatment with nicotine during septic peritonitis results in a reduction of local and systemic inflammation, but increases lethality, because of a decrease in bacterial clearance and enhanced dissemination of bacteria.[17] A possible explanation is that host defense in septic peritonitis is a delicate balance between pro-inflammatory pathways intended to eliminate bacteria and anti-inflammatory pathways intended to prevent systemic inflammation, and any imbalance in pro- or anti-inflammatory mediators might be harmful. Accordingly, 20 hours after E. coli challenge, α7nAChR knock-out mice display an accelerated bacterial clearance as compared with wild-type mice. As a result of reduced bacterial loads, α7 nAChR knock-out mice have reduced numbers of infiltrating neutrophils and lower circulating cytokine levels at that time point.[18]

Studies demonstrate that inflammatory response in sepsis can be controlled *via* activation of the central cholinergic system. CNI-1493 administered *via* the intracerebroventricular route is more effective in suppressing LPS-induced TNF-α release and shock as compared with intravenous dosage. Such protective effects require an intact vagus nerve. Similarly, intravenously administered oxytocin can reduce the neuroendocrine and cytokine response to bacterial LPS by increasing the excitability of central vagal neurons and CAP.[19] Moreover, ghrelin, a novel orexigenic hormone, is produced predominately in the gastrointestinal system, and intravenously administration of exogenous ghrelin markedly reduces TNF-α and IL-6 levels through central activation of vagus nerve in sepsis.[20]

Cholinesterase inhibitors can also control the inflammatory response in experimental sepsis. Cholinesterase inhibition significantly improves survival if administered directly after induction of sepsis, but no trend to a reduction in mortality is achieved when treatment is delayed.[21] Moreover, the mortality is increased when higher doses of cholinesterase inhibitors are administered. These drugs presumably act by raising and prolonging the profile of acetylcholine *via* an inhibitory effect on the esterase.

Patients with sepsis display evidence for activation of the coagulation system, which in its most extreme form can result in disseminated intravascular coagulation. Endothelial cell activation is critical in this process. Evidence shows that cholinergic stimulation blocks endothelial cell activation and leukocyte recruitment; VNS inhibits activation of coagulation and fibrinolysis during endotoxemia in rats. Therefore, CAP has impacts on inflammatory process and the coagulant/anticoagulant balance in sepsis.

Hemorrhagic shock Hemorrhagic shock initiates an inflammatory response characterized by the upregulation of cytokine expression. These changes contribute to end-organ damage and resultant dysfunction after shock. Application of constant voltage pulses to the caudal vagus ends increases survival time, reverts the marked hypotension, blunts the augmented NF-κB activity and inhibits TNF-α synthesis in animal models of acute hypovolemic hemorrhage. The protective effects of VNS are mediated through nicotinic receptors.[22] The melanocortin ACTH (1-24) suppresses the NF-κB-dependent systemic inflammatory response triggered by hemorrhage, and reverses shock condition. And bilateral cervical vagotomy prevents the life-saving effect of ACTH (1-24).[23] The data suggest that ACTH(1-24) acts by brain activation of the CAP. It has been demonstrated that ingestion of dietary fat stimulates cholecystokinin receptors and leads to attenuation of the inflammatory response. Vagotomy and administration of antagonists for cholecystokinin as well as nicotinic receptors significantly blunt the inhibitory effect of high-fat enteral nutrition on hemorrhage-induced TNF-α and IL-6 release.[24] These data revealed that nutritional stimulation of cholecystokinin receptors inhibits inflammation via the vagus nerve. Therefore, intestinal hyporesponsiveness to dietary antigens may be associated with tonic anti-inflammatory effects of vagus nerve.

Ischemia-reperfusion injury Ischemia-reperfusion injury is a pathologic event characterized by tissue damage. It is mediated by TNF-α and other cytokines that activate complement and proteases and stimulate fibrinolysis, degranulation of white blood cells, and free radical production. During reperfusion injury in a standard model of aortic occlusion, VNS significantly attenuates shock and inhibits TNF-α levels in serum, heart and liver.[25] Additionally, in a splanchnic artery occlusion shock model, VNS could increase survival rate, revert the marked hypotension, decrease hepatic TNF-α mRNA, reduce plasma TNF-α, ameliorate leukopenia, and decrease leukocyte accumulation in ileum and lungs. Chlorisondamine, an irreversible peripheral nicotinic receptor-blocking agent, can abate the effects of VNS. Thus, the anti-shock mechanism of VNS might be that acetylcholine, released by vagal ends, diffuses in liver resident macrophages before being completely hydrolyzed and blunts the inflammatory cascade. Furthermore, VNS strongly decreases the high incidence of severe arrhythmias and lethality, reduces the increase in free radical blood levels and left-ventricle histologic alterations during an experimental model of ischemic heart disease. Treatment with the melanocortin produces the same protective effects of VNS through brain activation of CAP.[26] Cholinergic agonists also have a protective effect in ischaemia/reperfusion injury. For instance, nicotine or GTS-21 can attenuate renal dysfunction, tubular necrosis, TNF-α production and leukocyte infiltration induced by renal ischemia.[27] The reduction in α7 nAChR expression following nicotine

treatment may represent an important protective mechanism. The increase in α 7 protein after renal ischaemia/reperfusion injury may augment the calcium influx, which is a major mechanism of cell injury and death during ischaemia/reperfusion injury.[28] Recent study reveals that ghrelin administration down-regulates pro-inflammatory cytokine expression, reduces neutrophil infiltration, ameliorates intestinal barrier dysfunction, attenuates organ injury and improves survival after gut ischaemia/reperfusion injury. Beneficial effects of ghrelin on gut ischaemia/reperfusion injury are mediated through the central activation of the CAP.[29] Finally, Huperzine A, a selective acetylcholinesterase inhibitor, exhibits anti-inflammatory and neuroprotective effects against transient focal cerebral ischemia-induced brain injury created by middle cerebral artery occlusion. The protection mechanism involves the inhibition of acetylcholinesterase activity and activation of nAChR in central.[30]

Pancreatitis A lot of clinical and experimental evidences suggest that cytokines play a key role in the pathogenesis of local and systemic complications of acute pancreatitis. In mouse model of pancreatitis induced by cerulein, vagotomy exacerbates inflammation and this effect is counteracted by pretreatment with nicotine or GTS-12.[31] These results imply an important role for the nAChR α7 subunit in mediating the cholinergic anti-inflammatory effect.

Ileus Ileus is a complication of abdominal surgery or trauma characterized by gastrointestinal hypomotility and delayed gastric emptying. An enteric molecular inflammatory response impairs local neuromuscular function, activates neurogenic inhibitory pathways and inhibits motility of the entire gastrointestinal tract. In animal models of postoperative ileus, VNS or administration of nicotine attenuates hypomotility and reduces cytokine production through an α7 nAChR-mediated activation of JAK2/STAT3 pathway.[5] Similarly, pretreatment with AR-R17779, a selective α7 nAChR agonist, could prevent delayed gastric emptying and reduce inflammatory cell recruitment. Moreover, AR-R17779 and nicotine reduce pro-inflammatory cytokine formation in peritoneal macrophages and the latter is more effective, which implies that nicotinic inhibition of macrophage activation may involve other receptors in addition to α 7 nAChR.[32]

Colitis Colitis are characterized by pro-inflammatory cytokines, tissue damage and loss of neuron in inflamed mucosa, implying that CAP may be destroyed during the process of inflammatory response.

Cholinergic agonist could inhibit colonic inflammatory response. For instance, nicotine decreases the levels of pro-inflammatory mediator in colonic mucosa during experimental colitis. It also has beneficial effects on the patients with ulcerative colitis, but clinical usage of nicotine is limited because of its side effects. Anabaseine, a cholinergic agonist, could reduce tissue damage and decrease myeloperoxidase activity and TNF-α level in colon of mice with colitis, whereas nicotinic receptor antagonist could reverse the above-mentioned effects.[33] Moreover, depression induced in mice with previously established colitis, can exacerbate the reactivation of colitis and the harmful effects of depression can be attenuated by choline-chloride, a specific α-7 agonist.[34] The vagus nerve also has a tonic efferent cholinergic anti-inflammatory effect in acute experimental colitis[35] and chronic colitis[36]. Colitis induced by dextran sodium sulfate is more severe in vagotomized mice compared with sham-operated mice. As the vagus nerve does not innervate the distal colon and rectum, the areas are usually affected in patients with inflammatory bowel diseases. The anti-inflammatory effect of vagus nerve can be possibly clarified by the role of the spleen in exerting the anti-inflammatory effect of vagus nerve signaling or by changes in autonomic (para)sympathetic balance.[34] Furthermore, acetylcholinesterase inhibitors could effectively modulate the acute colonic inflammation associated with dinitrobenzene sulfonic acid-induced colitis.

Interestingly, vagotomy has little pro-inflammatory effects in the chronic colitis induced by transfer of $CD_4^+CD62L^+$ T lymphocytes into severe combined immunodeficiency disease mice. That may be due to the unknown effects of vagus nerve on T cells.[37] In another study, experimental colitis was aggravated in nAChR α5 subunit deficient mice, suggesting that not only the α7 nAchR, but also other nAChR subunits could participate in the vagus modulation of colitis in mice.[38]

Alzheimer disease Alzheimer disease is characterized by inflammatory process in the senile plaques and surrounding glia, with enhanced expression of acute phase proteins, in which a local inflammatory response is sustained by microglial cells. Activated microglial cells

could cause neuronal damage *via* liberation of free radicals as well as cytokines and toxic factors. In an *in vitro* study, it revealed that activation of microglial cells by nicotine dose-dependently reduces the LPS-induced release of TNF-α. Nicotine can also activate microglial α7 nAChRs, drive a phospholipase C/IP3 pathway and modulate cell activation toward a neuroprotective role. Moreover, nicotine decreases β-amyloid peptide, a hallmark of Alzheimer disease, *via* the activation of α7 nAChRs through mitogen-activated protein kinase, NF-κB and c-myc pathways.[39] Epidemiological studies indicate that nicotine might be protective against the development of Alzheimer disease. Nicotine-mediated neuroprotection in these studies provide a mechanistic basis for the potential development of drug target for treating Alzheimer disease.

Myocardial infarction Within minutes of acute myocardial infarction, pro-inflammatory cytokines increase in the brain, heart, and plasma. Francis et al[40] noted that vagotomy one hour before ventricular ischaemia in rats had anti-inflammatory effects, e.g. diminishing the post-infarction TNF-α increase in myocardium. A possible explanation is that vagotomy has substantial effects on heart rate and haemodynamics which may have determined the early response to vagotomy. The other explanation might be the distinction between acute (within minutes) and chronic (within days and months) effects of vagotomy in animals.[41] It is demonstrated that in a rat model of endotoxaemia, vagotomy increases plasma TNF-α levels when performed 3 days before LPS administration. In contrast, vagotomy paradoxically diminishes plasma TNF-α levels when performed 30 minutes directly before LPS administration (which seems to be due to the release of acetylcholine by nerve endings during perivagotomy mechanical manipulations on vagal nerve).

Arthritis A possible anti-inflammatory role for CAP in arthritis was suggested by studies that showed an anti-inflammatory effect of cholinergic stimulation (including VNS, cholinergic agonists) on carrageenan-induced paw inflammation and carrageenan air pouch model.[42] Subsequent study showed that unilateral cervical vagotomy exacerbated collagen-induced arthritis and the treatment with an α7 nAChR agonist (AR-R17779) ameliorated the disease.[43] Consistently, a marked increase was found in clinical arthritis scores and synovial inflammation in α7-deficient mice compared with wild-type littermates in both acute and chronic phase of the disease. Underscoring the importance of α7 nAChR in rheumatoid arthritis, nicotine and α7 nAChR-specific agonists (PNU-282987) can modulate the inflammatory response of fibroblast-like synoviocytes *in vitro* for the patients with rheumatoid arthritis.[44] Moreover, *in vivo* study showed that AR-R17779 had an anti-inflammatory effect on collagen-induced arthritis.[43] AR-R17779 poorly crosses the blood-brain barrier, suggesting that the anti-inflammatory effects of this agonist are probably due to the peripheral stimulation of α7 nAChR. It has recently been shown that VNS can suppress the development of collagen-induced arthritis in rats.[45] The reduction of inflammation observed following VNS may be attributable to the down-regulation of cytokine production by the reticuloendothelial system and redirection of leukocytes trafficking away from the periphery.

Others In Fas-induced fulminant hepatitis, vagotomy triggers exacerbation of hepatitis, whereas supplementation with α7 nAChR agonists dose-dependently inhibits this detrimental effect of vagotomy. Thus, the hepatic vagus nerve appears to play an important role in attenuating Fas-induced hepatocyte apoptosis through α7 nAChR.[46] In an animal model of ricin poisoning, nicotine administration could attenuate cellular oxidative stress, reduce serum levels of TNF-α and markers of kidney as well as liver dysfunction, and decrease mortality.[47] Moreover, the combined signaling by the nicotine and estrogen synergistically reduces experimental chronic bladder inflammation, which can be measured by macrophage infiltration and up-regulation of IL-6 expression in the bladder.[48] Finally, VNS is followed by anti-inflammatory reactions within myocardial tissue in a canine model of chronic heart failure.

Relationship between vagus nerve activity and inflammatory diseases

Clinical research implicates a significant correlation between depressed vagus nerve activity and increased severity of inflammatory diseases including sepsis, rheumatoid arthritis, lupus, sarcoidosis, inflammatory bowel diseases. For example, HMGB1 is a cytokine implicated in the pathogenesis of rheumatoid arthritis. Serum HMGB1 levels in patients with rheumatoid arthritis were inversely related to RR interval variability (RRV, an index of vagus nerve activity).[49] Lower vagal nerve activity also contribute to the increased incidence of

sudden death observed in rheumatoid arthritis. Data also show that Alzheimer disease and other brain degenerative disorders are characterized by cholinergic deficiency and decreased vagus nerve activity. In addition, vagally mediated heart rate variability is inversely correlated with inflammatory markers. For instance, an inverse relationship in apparently healthy humans between an index of vagally mediated cardiac activity and C-reactive protein *in vivo* has been demonstrated after controlling a wide range of covariates including an index of sympathetic nervous system activity.[50] H. pylori infection has protective effects against gastroesophageal reflux disease and one mechanism might be that H. pylori infection in gastric mucosa induces a T helper 1-like immune response and production of pro-inflammatory cytokines which elevate the vagus nerve activity to alleviate oesophageal inflammation, and lead to a decrease in reflux-induced oesophageal injury and dysfunction.[51] Interestingly, increased vagal tone is associated with increased mortality and poor outcome in patients with traumatic brain injury. Sustained effects of CAP in patients with traumatic brain injury could severely impair the body's ability to combat infection and cause immune paralysis, which is responsible for the increased mortality.[52] Finally, to be emphasized, a causal relationship between depressed vagus nerve activity and increased inflammatory disease severity has not been established.

Various therapeutics of inflammatory diseases could increase vagal nerve activity. For example, acupuncture, an adjunct therapy of conventional medical treatment for a number of chronic inflammatory and autoimmune diseases can calm the mind, slow down the heart rate and stimulate gastric mobility.[53] The systemic anti-inflammatory actions of traditional and electro-acupuncture are directly or indirectly mediated by the efferent vagus nerve activation and inflammatory macrophage deactivation. Meanwhile, complex behavior originated in higher brain centers, such as hypnosis, meditation, prayer, guided imagery, biofeedback, and the placebo effect, can directly influence outflow through the vagus nerve and exert anti-inflammatory effects. Exercise reduces levels of TNF-α and other cytokines, increases vagus nerve activity and protection against cardiovascular disease and type II diabetes.[54] Conversely, obesity is characterized by diminished vagus nerve output and elevated cytokine levels, both of which involve in the pathogenesis of insulin resistance and atherosclerosis. Clinical studies have denmonstrated that high-fat nutrition could effectively increase vagus nerve activity, reduce the production of inflammatory mediators and improve inflammatory bowel disease, rheumatoid arthritis and cardiovascular diseases. These effects of high-fat nutrition may be related to the activation of CAP, just like the anti-inflammatory mechanism in animals.

Conclusion

The diffusible anti-inflammatory network, which includes glucocorticoids, anti-inflammatory cytokines and other humoral mediators, is concentration gradient-dependent, slow and nonintegrated. In contrast, CAP is discrete and localized in tissues where invasion and injury are typically originated. Compared with the routine biological pace of a typical, diffusible inflammatory response (hours to days), neural signaling is lightning fast. In addition, this pathway can inhibit various pro-inflammatory cytokines and overcome the limitation of anti-inflammatory agents targeting single mediator. Thus, it might be beneficial to sepsis in which multiple inflammatory mediators participate in the excessive inflammatory response. VNS has shown better control of depression with marginal side effects and it might provide an advantage for the treatment of chronic inflammatory disorders such as rheumatoid arthritis. α7 nAChR is a pharmacological target for inflammation in the CAP. Future studies are needed to evaluate the potential use of specific α7 nAChR agonists and determine their effects on physiological variables including blood pressure, respiratory status and renal function.

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